International Journal of Anatomy and Research,
Int J Anat Res 2015, Vol 3(2):1168-72. ISSN 2321- 4287
DOI: http://dx.doi.org/10.16965/ijar.2015.193

Original Article

TRACE ELEMENT AND LIGHT MICROSCOPIC STUDIES ON TESTIS OF ALBINO RATS TREATED WITH SILDENAFIL CITRATE (CAVERTA)

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ABSTRACT

Background: Caverta (Sildenafil citrate), an oral therapy for Erectile Dysfunction (ED), being the citrate salt of Sildenafil, is a selective inhibitor of cGMP- specific phosphodiestrase type (PDE5).

Aim: To study the drug- induced (i) changes in the trace element content of Testis and (ii) changes in the histoarchitecture of Testis of the experimental Albino rats.

Materials and Methods: For the present study, totally 48 animals were selected on weight basis and divided into 8 groups (S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_8) with six animals in each group. Control animals (S_1) were fed with conductivity water while the experimental animals (S_2, S_3, S_4 and S_5) were treated with a single dose of Caverta (@ 1µg/g body weight). Control animals were sacrificed at zero hour while the experimental animals (S_2, S_3, S_4 and S_5) were sacrificed after 1 hour, 2½ hours, 4 hours and 24 hours of drug administration respectively S_6, S_7 and S_8 group of animals were fed with a single dose of the chosen drug (@ 1µg/g body weight) daily for all the 15, 30 and 45 days respectively. These animals were sacrificed after 4 hours of the last dosage. Vertical ventral midline incision was made in the abdominal wall to collect the left and right Testes.

Results: The spectral analysis indicates that the long term Caverta treatment of Albino rats results in the accumulation of Iron and Copper levels accompanied by a depletion of Nickel levels in the Testis. The histological studies indicate that long term exposure of Testis to Caverta leads to distorted histoarchitecture of the seminiferous tubules, interstitial space dilation and separation of Spermatogenic cells.

Conclusion: Long term Sildenafil Citrate (Caverta) treatment of Albino rats will bring in adverse effects and will completely alter the histoarchitecture of the Testis.

KEY WORDS: Trace Elements, Caverta, Testis, Histo architecture, Spermatogenic cells.

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Access this Article online

Quick Response code

DOI: 10.16965/ijar.2015.193

Web site: International Journal of Anatomy and Research
ISSN 2321-4287
www.ijmhr.org/ijar.htm

Received: 05 Jun 2015 Accepted: 19 Jun 2015
Peer Review: 05 Jun 2015 Published (O):30 Jun 2015
Revised: None Published (P):30 Jun 2015

INTRODUCTION

Erectile Dysfunction (ED) has been defined as the persistent inability of male to attain and maintain a penile erection sufficient to permit satisfactory sexual performance as part of overall process of male sexual function [1, 2]. It has been found that approximately 52% of the surveyed men aged between 40 and 70 had some degree of ED, with dysfunction being

moderate to complete in approximately half of the old men aged 70 years [3, 4].

Caverta (Sildenafil citrate), an oral therapy for ED, being the citrate salt of Sildenafil, is a selective inhibitor of cGMP- specific Phosphodiestrase type (PDE5). Even though Sildenafil citrate is well tolerated, it has some side effects.

Trace elements in animal nutrition have a threshold concentration and play a crucial role in the growth and metabolism of that animal. Iron- induced cellular dysfunction and Lipid peroxidation, Copper- induced liver and kidney dysfunctions, Zinc- induced growth retardation and hypogonadism and Selenium- induced human infertility are a very few illustrations to stress the biological significance of these trace elements.

Therefore, it is of interest to carry out, in the present investigation, (i) trace element analysis using ICP- AES technique to notice the changes, if any, in the level of these elements and (ii) Light microscopic analysis to notice the changes, if any, in the structure of the organ due to the drug (Caverta) administration.

MATERIALS AND METHODS

For the present study, totally 48 animals (Wistar male healthy Albino rats) were selected on weight basis and divided into 8 groups (S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_8) with six animals in each group. Control animals (S_1) were fed with conductivity water while the experimental animals (S_2, S_3, S_4 and S_5) were treated with a single dose of the chosen drug (@ 1µg/g body weight). Control animals were sacrificed at zero hour while the experimental animals (S_2, S_3, S_4 and S_5) were sacrificed after 1 hour, 2½ hours, 4 hours and 24 hours of drug administration respectively. S_6, S_7 and S_8 group of animals were fed with a single dose of the chosen drug (@ 1µg/g body weight) daily for all the 15, 30 and 45 days respectively. These animals were sacrificed after 4 hours of the last dosage. 'Drug' here refers to the appropriate dosage of Sildenafil citrate (Caverta) procured from the market.

These animals were acclimatized for a period of seven days before starting the study. Standard experimental conditions such as temperature (24±2°C), humidity (60- 70%) and 12 hours of light/ dark cycle were maintained. Food and water were allowed *adlibitum* under strict hygienic conditions. Chloroform anaesthesia was used and a vertical ventral midline incision was made in the abdominal wall to collect both the left and right Testis samples.

Sample preparation:

(i) ICP- AES Studies: The collected Testes samples were first over dried and then, ashed. The ashed samples were dissolved in 20% HNO_3 and the filtrate was used for the present study. Using the standard spectral grade solutions, the quantitative estimation of Iron (Fe), Copper (Cu) and Nickel (Ni) present in Testis samples of the experimental animals was made by employing Inductively Coupled Plasma- Atomic Emission Spectrometer (ICP- AES) [JY- 24 ICP- AES]

(ii) Light microscopic studies: The collected Testis samples were preserved in 10% formalin, processed and stained with Eosin and Haematoxylin stains.

RESULTS AND DISCUSSION

(i) ICP- AES Spectral Studies: The outcome of the present spectral analysis has been tabulated in Table 1. A heavy loading of Iron and Copper has been noticed for the Testis samples of the long term Caverta treated (15, 30 and 45 days) Albino rats. Though the level of Nickel has been found to be almost the same for all the long term animals, it is much less than that of the control (0 hr.) samples.

Table 1: Representing the ICP- AES analysis of Testis of Caverta treated Albino rats.

Element	Concentration (ppm) [a,b]							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Iron (Fe)	10.15 ± 0.06	13.37 ± 0.78	11.32 ± 0.28	14.16 ± 1.69	10.18 ± 0.17	16.29 ± 2.38	19.37 ± 0.92	15.76 ± 0.96
Copper (Cu)	0.27 ± 0.01	0.21 ± 0.01	0.14 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.51 ± 0.02	0.9 ± 0.04	0.35 ± 0
Nickel (Ni)	0.28 ± 0	0.35 ± 0.01	0.25 ± 0.02	0.32 ± 0.02	0.35 ± 0.01	0.18 ± 0.03	0.17 ± 0.02	0.19 ± 0.01

a-Based on triplicates
b- Based on dry matter basis
S_1-Control (0 hr.)
S_2- Drug treated (1 hr.)
S_3- Drug treated (2.5 hrs.)
S_4- Drug treated (4 hrs.)
S_5- Drug treated (24 hrs.)
S_6- Drug treated (15 days)
S_7- Drug treated (30 days)
S_8- Drug treated (45 days)

The results of the present spectral analysis of Testis of Caverta treated Albino rats can be discussed in detail as follows:

The role of minerals in reproduction has been studied by Mc Clure [5] for cattle. Many trace elements function as co- factors and activators of enzymatic activities in carbohydrate metabolism [6].

Change in Iron content in Testis may be correlated to peroxidase activity. Hence, an increase in Iron content will certainly increase the peroxidase activity. Though the content of Iron is high in Testis samples of long- term Caverta treated animals, it starts decreasing for 45 days samples. Low level of Iron has been reported to be responsible for the infertility in Heifers [7, 8]. Therefore, Caverta treatment of Albino rats, on long term basis, is to be done with proper care as it may lead to the decrease in Iron content.

Though the earlier workers [9, 10] have reported Copper to play an important role in the fertility of animals, role of Copper in association with reproduction, at biochemical level, is yet to be established [11]. Copper toxicity, as noticed in the present study, may be considered as a responsible factor for growth retardation and for the reported mild to moderate side- effects such as irritation and nausea during the long term usage of the drug [12]. Perturbance in Copper level, as detected in the present study may lead to alterations in the cell membrane integrity, enzyme inhibition and reduced stability of DNA. This situation may ultimately result in potential damage to the tissue [13].

Nickel deficiency has been found to lead to decreased growth. Moreover, the analysis of different parts of the body showed that Nickel deficient animals were not only poor in Nickel and Calcium but also in Zinc [12]. Therefore, the decreasing trend in Nickel content in Testis, as observed in the case of the long-term drug treated animals, may be considered to lead to Zinc deficiency. Zinc is important for spermatogenesis and epididymal secretion and the probable site of action of the metal has been primary spermatocytes, spermatids, sertoli cells and Leydig cells [14].

Thus, it is concluded from the present spectral studies on Testis that the long- term Caverta treatment of Albino rats may result in altered situation in trace element environment which may culminate in enhanced peroxidase activity, growth retardation, tissue damage and structural deformities. Besides, the spermatogenesis and the epididymal secretion of Testis may also get drastically affected due to the Caverta induced alterations in mineral status of Testis.

(ii) Light Microscopic Studies: The Histological observations, made in using Light Microscope as an analytical probe, have been depicted as figures (Fig. 1 to Fig. 4). A close scrutiny of the Figures clearly portrays that the long- term drug treatment results in the thickening of basement membrane, separation of Spermatogenic cells, marked dilation of interstitial space and prominent distortion of Histoarchitecture of the seminiferous tubules.

Fig. 1: Section of Testis of Albino rats [S_1 (0 hr.)] showing (1) Seminiferous tubule, (2) Interstitial space and (3) Spermatogenic cells. Haematoxylin- eosin x 100.

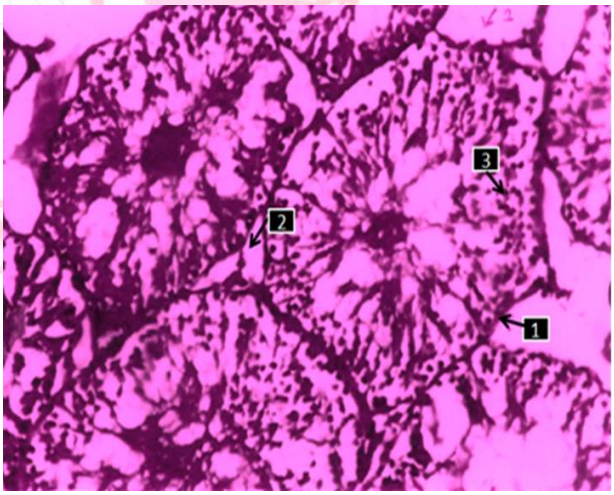


Fig. 2: Section of Testis of Caverta treated Albino rat [S_6 (15 days)] showing (1) Seminiferous tubules, (2) Marked dilation of interstitial space and (3) Marked dilation of blood vessel. Haematoxylin- eosin x 100.

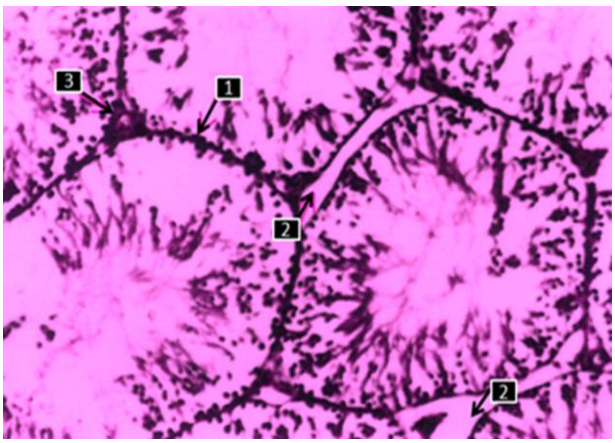


Fig. 3: Section of Testis of Caverta treated Albino rat [S_7 (30 days)] showing (1) Basement membrane, (2) Separation of Spermatogenic cells and (3) Oedematous seminiferous tubule. Haematoxylin- eosin x 100.

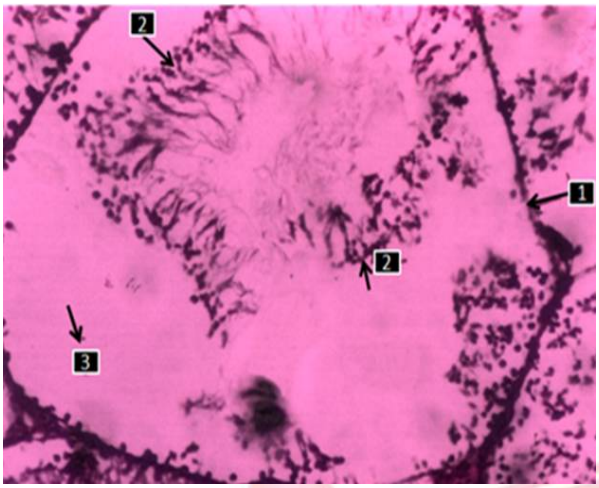
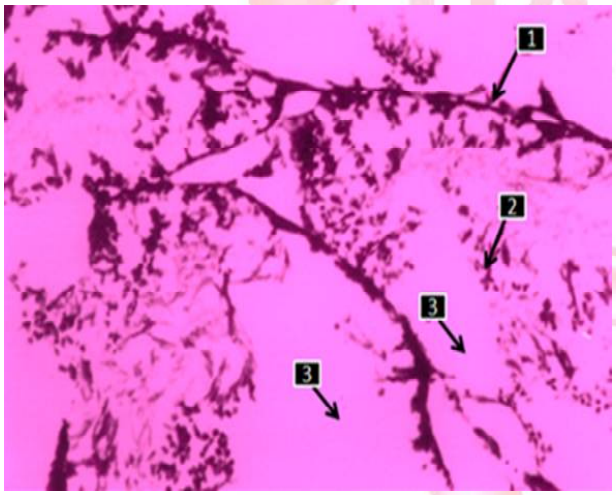


Fig. 4: Section of Testis of Caverta treated Albino rat [S_8 (45 days)] showing (1) Distorted histoarchitecture of the basement membrane, (2) Spermatogenic cells and (3) Oedematous seminiferous tubule. Haematoxylin- eosin x 100.



The above Histological observations made on Testis of drug treated Albino rats can be discussed as follows:

Robbins and Cotran [15] in their classical text book, have stated that, "In diabetes mellitus, the basement membranes are leakier than normal, so that microangiopathic vessels are absolutely permeable to plasma proteins; insudation of these proteins has been thought to contribute to the basement membrane thickening." From the ICP- AES studies, as carried out in the present investigation, it is quite obvious that the Caverta administration results in disturbed metabolism. Moreover, the basement membrane thickening in Testis of the experi-mental animals was observed in the present study as a constant qualitative change. Therefore, the Caverta induced changes in the metabolism of Albino rats are to be observed as similar to that of drug-induced diabetes mellitus. As such, the thickening of the basement may be visualized as the result of long-term Sildenafil citrate administration.

From the Histological studies, it has been observed that there is an increase in the intertubular area which may be due to the intertubular oedema in the Testis. For all the experimental animals (especially long –term drug treated), the seminiferous tubules have been observed to be widely separated from each other. It may be inferred that this state of seminiferous tubules is due to the intertubular oedema which disturbs the nutrition supply to the seminiferous epithelium and may ultimately result in lysis of Spermatogenic cells. It has been reported by earlier workers [16] that spermatids are the most sensitive, among the Spermato-genic cells to any physiological alterations. Therefore, the metabolic changes as observed in the present study may induce qualitative and quantitative changes in spermatids.

The interstitial oedema may make the interstitial cells inefficient in their functions, resulting in impotency. As Sildenafil citrate (Caverta) is exclusively used for the treatment of Erectile dysfunction and impotency, the inference that long- term (Caverta) treatment may bring about the reversal effect is to be closely scrutinised.

Similar histological changes indicating the accumulation of oedematous fluids and distorted histoarchitecture due to drug treatment have been noticed even in the case of Testis of rats treated with the leaf powder of Azadirachta indica [17] and Andrographics poniculata (Nees) [18], the latter being known for its antifertility activities.

CONCLUSION

It may be concluded, from the present trace el-ement and histological studies, that the long term Sildenafil citrate (Caverta) treatment of Albino rats will certainly bring in adverse effects on metabolism and completely alters the histoarchitecture of Testis.

ACKNOWLEDGEMENT: The authors sincerely acknowledge the kind help and co- operation rendered by the authorities of Sri Manakula Vinayagar Medical College and Hospital (SMVMC&H) and Annamalai University (AU).

Conflicts of Interests: None

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How to cite this article:

K. V. P. Suryakumari, R. Uday Kumar, D. Savitha. TRACE ELEMENT AND LIGHT MICROSCOPIC STUDIES ON TESTIS OF ALBINO RATS TREATED WITH SILDENAFIL CITRATE (CAVERTA). Int J Anat Res 2015;3(2):1168-72. **DOI:** 10.16965/ijar.2015.193